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Research Article

A redescription and new host record for *Dactylogyrus nasutai* Narba and Wangchu, 2015 (Platyhelminthes, Monogenea) from Arunachal Pradesh, India using high-resolution microscopy imaging

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Abstract
Dactylogyrus spp. are monogenean ectoparasites that infect economically important fishes in aquaculture worldwide. Currently, over 900 nominal species of *Dactylogyrus* are known. *Dactylogyrus nasutai*, originally described from the cyprinid fish *Garra nasuta* in Arunachal Pradesh, India, is redescribed and illustrated based on high-resolution microscopy imaging of specimens collected from the gills of *Garra annandalei*. The results reveal previously undescribed morphological traits, such as the accessory piece having a slender tube with serrations along its length and a prominent hook-shaped distal extension and dorsal anchors with notable depressions at the bases and lateral expansion in their shafts. *Garra annandalei* is the first host record for a monogenean parasite and a new host record for *Dactylogyrus nasutai*. The results of this study clarify the morpho-taxonomic details that will allow an unambiguous identification of *Dactylogyrus nasutai*.

Keywords: High-resolution microscopy imaging, Monogenea, *Dactylogyrus nasutai*, River Tirap, Arunachal Pradesh

INTRODUCTION

Arunachal Pradesh is a state in India's far northeastern region. It is a part of the Indo-Myanmar biodiversity hotspot (Myers *et al*., 2000) and one of the 200 globally significant ecoregions (Olson and Dinerstein, 1998). With an estimated 213 plus fish species, most of which are endemic to the state (Bagra *et al*., 2009), Arunachal Pradesh has 23.43 percent of all Indian freshwater fish species. *Garra annandalei* Hora, 1921 is a freshwater, benthopelagic cyprinid fish species found in highland streams of Asia (India, eastern Nepal, Bangladesh, and Bhutan) (Froese and Pauly, 2022). In Arunachal Pradesh, this species is a locally popular food fish (Darshan *et al*., 2019).

Dactylogyrus Diesing, 1850 is the largest helminth genus comprising over 900 nominal species worldwide (Gibson et al. 1996). *Dactylogyrus nasutai* was described by Narba and Wangchu (2015) from the gill lamellae of a cyprinid fish *Garra nasuta* (McClelland, 1838) collected from the River Kael, Yachuli, in Arunachal Pradesh. The original description of the species was, however, scant and lacked some important diagnostic characters. During an ongoing study of monogenean parasites from Arunachal Pradesh, specimens of *D. nasutai* were recovered from the gill lamellae of *Garra annandalei*. The goal of this study was to provide a comprehensive redescription of *D. nasutai* using high-resolution imaging by light microscopic examination of sclerotized structures to aid in the identification and

description of the parasite species.

MATERIALS AND METHODS

Experiments were conducted in accordance with institutional guidelines for animal care. In January 2022, a total of 10 dead specimens of *G*. *annandalei* were collected from the River Tirap in Tirap District of Arunachal Pradesh, India, using the stone piling method. The specific identity and classification of fish was determined following the keys provided by Talwar and Jhingran (1991). Their gill arches were removed and immediately fixed and preserved in 5% formalin and later examined for monogenean parasites under a stereomicroscope. *Dactylogyrus* specimens were detached from the gills using fine needles and mounted on glass slides following Wangchu *et al.* (2017). The mounted specimens were examined, photographed, and measured (in micrometers) using a light microscope equipped with phase-contrast optics, a digital camera and image analysis software (Leica DM4B, Leica Microsystems Ltd.). Parasites were identified based on the shape and size of the sclerotized parts of the haptor (anchors, connective bars, and hooks) and reproductive organs (male copulatory organ and vagina) following Gusev (1976). All structures, including the rings of the male copulatory apparatus and the vagina, were measured as a straight line extending between the two most distant sites of each structure. The numbering of hook pairs followed Kulwiec (1927). A drawing tube attached to the Olympus BX51 light microscope was used to prepare an illustration plate.

RESULTS

Monogenea Bychowsky, 1937
Dactylogyridea Bychowsky, 1937
Dactylogyridae Bychowsky, 1933
Dactylogyrus Diesing, 1850
Dactylogyrus nasutai Narba and Wangchu, 2015 (Figs. 1-3)
Type host and locality: *Garra nasuta* (McClelland, 1838); River Kael, Yachuli, Arunachal Pradesh, India (29°30' N; 97°30' E)
Present record: *Garra annandalei* Hora, 1921 (Cypriniformes: Cyprinidae); River Tirap, Tirap, Arunachal Pradesh, India (26°59' N; 95°32' E); January, 2022
Deposition of the type specimen: 4 vouchers (ZSI/W10939-42/1) in the Helminthological Collection of Zoological Survey of India (ZSI), Kolkata, India.
Redescription (based on 9 specimens; measurements presented as the mean followed by the range and number (n) of structures measured in parentheses)**:** With characters of the genus as defined by Diesing (1850). One pair of dorsal anchors, each with a prominent depression at the base and a lateral expansion in the shafts; total length 36 (34-41; n=8) long, inner root length 18 (16-22; *n*=8), outer root length 4 (3-4; *n*=8), point 10 (9-10; n=7) long, recurved, extending beyond the level of tip of inner root. Dorsal bar straight, robust, with lobed ends, 25(24-25; n=7) long, 3(3-4; n=6) wide. Ventral bar reduced, stick-shaped with a median notch and tapering ends, 11(8-13; n=5) long. Seven pairs of similar hooks; each with a delicate point, an upright thumb, a shank comprised of 2 subunits (with the proximal subunit expanded), and an FH loop that does not extend to the point where the shank subunits meet. Hook lengths are as follows: pair i, 21 (20-22; *n* = 6); pair ii, 25 (24-26; *n* = 6); pair iii, 31 (30-32; *n* = 6); pair iv, 29 (28-30; *n* = 6); pair v, 23 (22-24; *n* = 6); pair vi, 18 (18-20; *n* = 6); pair vii, 20 (19-20); *n* = 6) long. The male copulatory organ comprised a proximally articulated copulatory tube and an accessory piece. Copulatory tube a coil of approximately 27 clockwise rings, 100 (78 -110; n=15) long. The accessory piece consists of a serrated rod lying within rings of the male copulatory tube; the proximal part articulates with the base of the copulatory tube, and the terminal part complex consists of membranous folds and a hook-shaped extension that guides the distal end of the copulatory tube; total length 93 (71-100; n=15). Vagina a coil of approximately 13 clockwise rings, total length 11 (9-14; n=15).

DISCUSSION

Historically, morphological characteristics of sclerotized parts of haptor and reproductive organs have been used to define the taxonomy of monogenean parasites (Malmberg 1970; Wong et al., 2006). The use of light microscopy imaging to decipher the details of the morphological properties of these sclerotized parts is also a well-established practice (Dos Santos et al., 2019).
Our specimens were identified as *D*. *nasutai* based on the morphology of their sclerotized structures, which were broadly similar to those described in the original description of *D*. *nasutai*. However, the use of high-resolution imaging by light microscopy revealed various new structures as well as differences in the comparative measurements of sclerotized structures (Figs. 1-3) (Table 1). Narba and Wangchu (2015) depicted the accessory piece as a thick rod of irregular shape, whereas we found it to be a slender tube with serrations running its length (Figs. 1 and 3). Similarly, the distal part of the accessory piece was found to have a complex structure consisting of membranous folds and a prominent hook-shaped extension to help guide the termination of the copulatory tube. Narba and Wangchu (2015) also overlooked the presence of prominent depressions at the base of dorsal anchors (Figs. 1-2); lateral expansion in the shafts of anchors was illustrat-

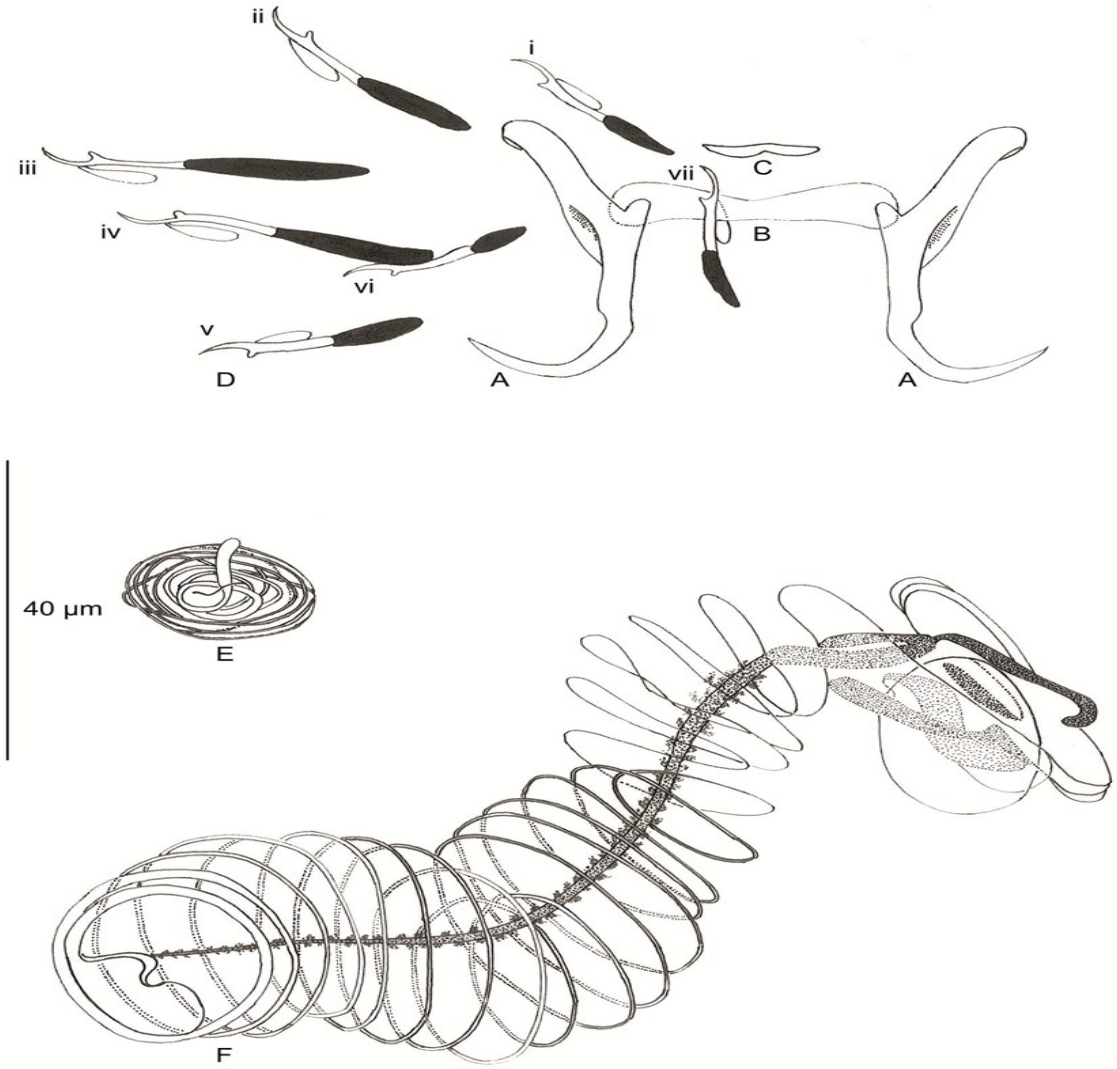

Fig. 1. *Line drawings of hard parts of haptor and reproductive organs of Dactylogyrus nasutai Narba and Wangchu, 2015 from Garra annandalei Hora, 1921. A. Dorsal anchor. B. Dorsal bar. C. Ventral bar. D. Hook (pairs i–vii). E. Vagina. F. Male copulatory organ. Scale bar = 40 µm*

ed but not mentioned in the description by these authors. The original description did not provide the length of all seven pairs of hooks as well; rather, it classified hook length into three arbitrary categories: one large (23 µm long), one median (18 µm), and 5 small (12 µm). The specimens in our collection, on the other hand, clearly had hooks of different lengths ranging from 18 to 29 µm (Table 1).

Narba and Wangchu (2015) were also unaware of the striking resemblance between the male copulatory organ and vagina of *D. nasutai* and that of *D. decaspirus* Guégan, Lambert and Euzet, 1988 (Syn. *Dactylogyrus* cf. senegalensis Paperna, 1979), which was described from the African carp *Labeo coubie* Ruppell, 1832 in Mali, Ghana, and Senegal. Both *D. nasutai* and *D. decaspirus* feature a copulatory tube comprised of rings and an accessory piece comprised of a serrated rod encircled by rings. *Dactylogyrus nasutai*, however, differs from *D. decaspirus* in that it has a copulatory tube with a larger number of rings (30 in *D. nasutai* versus 10 in *D. decaspirus*) and a longer length (100 µm in *D. nasutai* compared to 36 µm in *D. decaspirus*). *Dactylogyrus nasutai* further differs from *D. decaspirus* in having a prominent depression at the base of dorsal anchors (absent in *D. decaspirus*) and noticeably smaller anchors [36 (34-41) µm in *D. nasutai* n. sp. versus 55 (52–57) in *D. decaspirus*]. In addition, *D. decaspirus* is a parasite of *L. coubie* in Africa, while *D. nasutai* was collected from *G. nasuta* and *G. annandalei* in northeast India. The detailed comparative measurements of sclerotized structures of haptor and reproductive organs of *D. nasutai* collected from *Garra annandalei* (present study) and *Gara nasuta* (Narba and Wangchu, 2015) in India and *D. decaspirus* collected from *Labeo coubie* (Guégan et al., 1988) in Africa are presented in Table 1. Surprisingly, the male copulatory organ of *Dactylogyrus nasutai* also resembles very closely to a few monogenean genera other than *Dactylogyrus*,

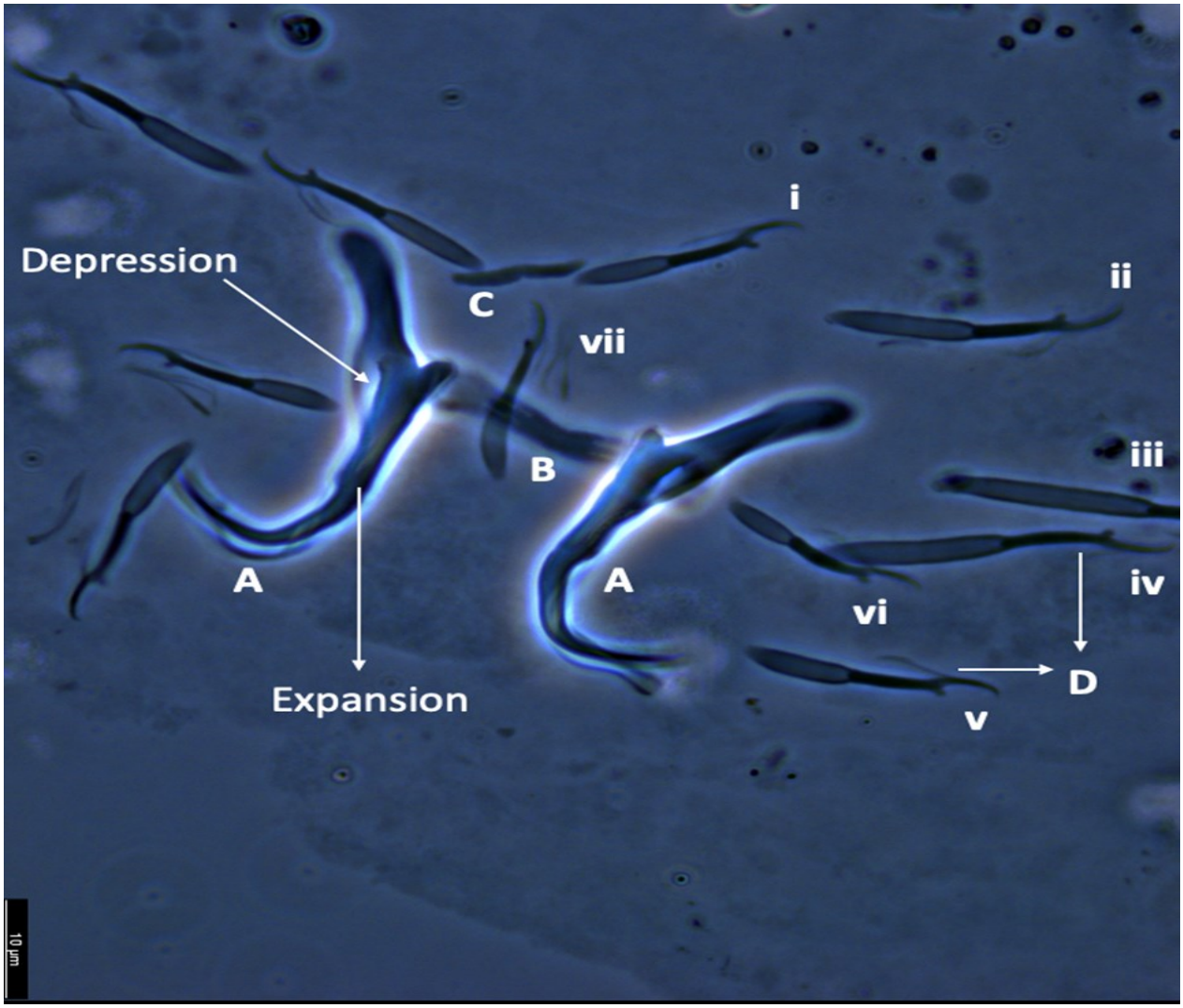

Fig. 2. *Phase-contrast photomicrograph of hard parts of haptor and reproductive organs of Dactylogyrus nasutai Narba and Wangchu, 2015 from Garra annandalei Hora, 1921. A. Dorsal anchor. B. Dorsal bar. C. Ventral bar. D. Hook (pairs i-vii). E. Scale bar = A–D: 10 µm*

most notably *Cichlidogyrus sanseoi* from *Hemichromis fasciatus* Peters, 1857 from Ivory Coast (Pariselle & Euzet, 2004), *Cichlidogyrus arthracanthus* from *Tilapia zillii* [now *Coptodon zillii* (Gervais, 1848)] from Israel (Paperna, 1960), and, to a lesser extent, *Thaparocleidus euzeti* from *Pangasius djambal* Bleeker, 1846 from Indonesia (Pariselle et al. 2002). The explanation(s) for this striking similarity between species of different genera are currently unknown.

Currently, 56 nominal species of *Dactylogyrus* are known from India (Wangchu *et al*., 2017). The majority of these species have been reported from north and south India, with only five being found in northeast India, all of them in Arunachal Pradesh: Narba and Wangchu (2015) described *D. nasutai*, and *D. yachulien-sis* from *G. nasuta* and *D. siangensis* from *Bangana dero* (Hamilton, 1822); Tripathi *et al*., (2016) recorded *D. sphyrnoides* Gussev, 1976 from *Tor tor* (Hamilton, 1822) and Wangchu *et al*., (2017) described *D. barnae* from *Barilius barna* [now Opsarius barna (Hamilton, 1822)]. This paper adds *G. annandalei* as the first host record for a monogenean parasite and, most likely, for any fish parasite. Arunachal Pradesh has a high level of hidden diversity of monogenean parasites owing to its diversified fish fauna (Tripathi, 2011). It is recommended that more fish species be sampled to fully realise the high diversity of monogenean parasites found in fish communities of Arunachal Pradesh.

Conclusion

Dactylogyrus nasutai from *G. nasuta* and *G. annandalei* in Arunachal Pradesh is a distinct species characterised and distinguished from its congeners by the presence of a significantly large number of sclerotized rings in its male copulatory organ and vagina. *Garra annandalei* is the first host record for a monogenean parasite and a new host record for *D. nasutai*. Additional fish species from Arunachal Pradesh need to be sampled to

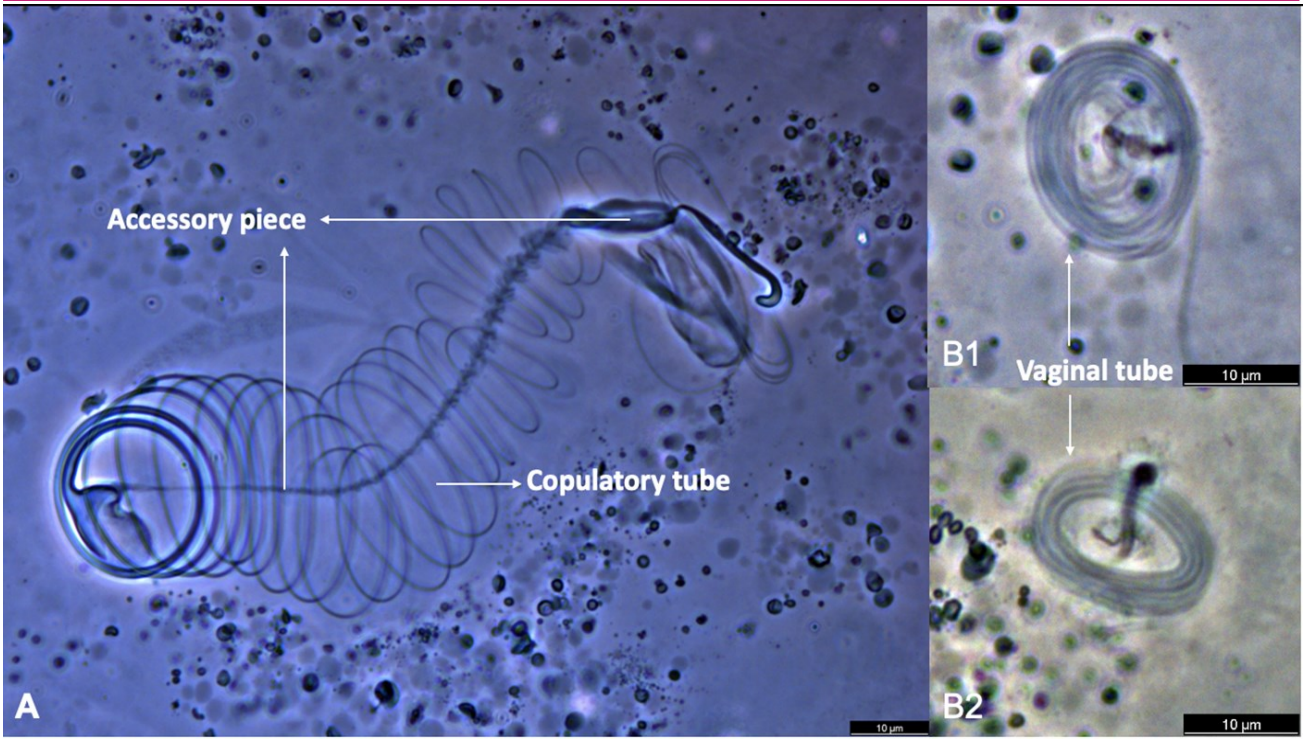

Fig. 3. *Phase-contrast photomicrographs of Dactylogyrus nasutai Narba and Wangchu, 2015 from Garra annandalei Hora, 1921. Hard parts of reproductive organs displaying sclerotized rings. A. Male copulatory organ, B1-B2. Different configurations of the vagina. Scale bar = 10 µm*

Table 1. Comparative measurements (in µm) of sclerotized parts of haptor and reproductive organs of *Dactylogyrus* spp.

Character	Measurements		
	Dactylogyrus nasutai Present study	**Dactylogyrus nasutai Narba and Wangchu (2015)**	**Dactylogyrus decaspirus Guégan, Lambert and Euzet (1988)**
Haptoral parts			
Dorsal anchor			
Total length	35 (34-41)	41 (40-41)	55 (52–57)
Inner root length	17	-	-
Outer root length	3	-	-
Point length	10 (9-10)	-	19 (18–20)
Dorsal bar			
Length	26(24-25)	25 (24-25)	25 (24–27)
Width	3 (3-4)	3 (2-3)	4 (4–5)
Ventral bar			
Length	11(8-13	8 (7-8)	9 (9–10)
Width	1 (1-2)	-	2 (2–3)
Hooks			
Pair i	21 (20-22)		25 (24–26)
Pair ii	25 (24-26)		28 (28–29)
Pair iii	31 (30-32)		29 (29–30)
Pair iv	29 (28-30)		26 (26–27)
Pair v	23 (22-24)		25 (24–26)
Pair vi	18 (18-20)		20 (19–20)
Pair vi	20 (19-20)		21 (20–22)
Reproductive organs			
Male copulatory organ			
Copulatory tube	100 (78-110)	59 (58-60)	36 (31–40)
Accessory piece	93 (71-100)	77 (70-80)	
Vagina			
Vaginal tube	13 (10-15)	13 (13-14)	-

(– show that these measurement values were not provided by the respective authors).

map the true diversity of their monogenean parasites.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical statement

Experiments were conducted in accordance with institutional guidelines for animal care.

Author contributions

DN and CM collected the host fish and parasite samples and performed the experiments; NA contributed reagents and/or materials and supervised the experiments; AT wrote and approved the final draft of the manuscript.

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